Original Communication

Effects of Vitamin E on Plasma Lipid Status and Oxidative Stress in Chinese Women with Metabolic Syndrome

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Abstract: Following the change of dietary structure and living style, metabolic syndrome (MetS) has become increasingly common in China, especially in women, who have abnormal plasma lipid profiles with increased levels of oxidative stress. Vitamin E (VitE) is a powerful chain-breaking antioxidant, which may be a protective factor against oxidative stress-related diseases. This study investigated the effects of three different dosages of tocopherol supplementation (100 IU /day, 200 IU /day, 300 IU / day) for 4 months in Chinese women with MetS. The plasma VitE concentrations increased significantly after the 4 months of supplementation ($p < 0.01$). The protective decreases in plasma total cholesterol were significant in 200 IU/day and 300 IU/day VitE groups ($p < 0.05$), but decreases in high-density lipoprotein cholesterol were also significant in all the supplementation groups ($p < 0.05$). Plasma triglycerides were unaltered ($p > 0.05$). The indicators of oxidative stress decreased substantially in all of the VitE supplementation groups: malondialdehyde (MDA) was reduced by nearly 50 percent (all groups, $p < 0.001$), erythrocyte hemolysis was decreased by nearly 40 percent (all groups, $p < 0.05$); among which the 300IU/day VitE group showed the most significant effect. However, the activity of superoxide dismutase (SOD) decreased after the trial ($p < 0.001$). VitE provided marked benefits in reducing oxidative stress levels and improving lipid status in women with MetS. Although no dose-effect relationship was observed, 300 IU VitE per day showed the optimal effect. Research is needed to identify potential protective mechanisms or utilization of vitamin E during MetS.

Key words: vitamin E, lipid, oxidative stress, hemolysis, metabolic syndrome.

Introduction

Following changes in dietary intakes and living style, metabolic syndrome (MetS) has become increasingly common in China, especially in women. Gu *et al.* [1] found that the prevalence of MetS was 9.8 % in men and 17.8 % in women in China. People with MetS, characterized by a group of metabolic risk factors, have been widely found to be at elevated risk of coronary heart disease and type 2 diabetes mellitus, in which increased levels of oxidative stress may play an important role. Several recent lines of evidence support the role of oxidative stress in the development of MetS. Cross-sectional data from 2,002 non-diabetic subjects of the community-based Framingham Offspring Study has shown that systemic oxidative stress is associated with insulin resistance [2], which plays a key role in the pathology of MetS, and which oxidative stress has been reported to elevate [3].

Reactive oxygen species (ROS) are constantly generated as a result of normal aerobic respiration, other metabolic processes, or external insult. It is well established that exposure of cells to ROS leads to oxidative modification of proteins, carbohydrates, nucleic acids, and lipids, which can contribute to the development of a number of diseases. Under normal conditions, antioxidant systems cope with elevated ROS. However, when ROS overwhelm the biological defenses of the cell, the result is oxidative stress [4]. Vitamin E (VitE), a powerful chain-breaking antioxidant, resides primarily in biological membranes and serves to protect membrane phospholipids from peroxidation. It has been shown that VitE significantly decreased biomarkers of oxidative stress [5] in intervention studies in humans. Epidemiological evidence suggests that VitE is a negative risk factor for oxidative stress-related diseases. A recent prospective study examining risk for prostate cancer showed a lower disease risk in subjects with higher plasma VitE levels [6]. But the results of studies with VitE intervention are not all the same and some of them are disappointing. In a follow-up study involving nearly 40,000 male health professionals with 4 years of follow-up, an inverse relationship was found between coronary artery disease and α-tocopherol intake [7]. Some authors discovered a paradoxical dose-dependent regulation of antioxidant capacity by VitE [8] and furthermore, it has been suggested that high doses of VitE may increase the risk of all-cause mortality [9] .

To our knowledge, the effect of VitE supplementation in subjects with MetS, which is also an oxidative stress-related disease, has not yet been performed, especially with different doses. In this study, we tested the hypothesis that vitamin E supplementation, in various dosages, could decrease the level of oxidative stress and ameliorate the altered lipid metabolism in MetS.

Subjects and methods

Study Subjects

The study received approval from the Ethics Committee of Qingdao University and all subjects gave written consent before their inclusion in the trial. One hundred non-smoking women who had MetS with an average age of 59.09 ± 3.53 (range, 55–69) and an average body mass index of 27.65 ± 2.37 (range, 25–35) were selected. The presence of MetS was defined according to the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III; ATP III) as the presence of three or more of the following risk factors: waist circumference greater than 102 cm in men or greater than 88 cm in women; serum triglyceride concentration of 1.7 mmol/L or greater; high-density lipoprotein (HDL)-cholesterol concentration of less than 1.0 mmol/L in men or less than 1.3 mmol/L in women; blood pressure 130/85 mm Hg or greater; or serum glucose concentration of 6.1 mmol/L or greater. Because the ATP III criteria for HDL cholesterol and waist circumference might not be appropriate for Asian populations, the criteria for MetS in Chinese women was adapted based on a recommended regional cutoff for HDL cholesterol of less than 1.0 mmol/L and waist circumference greater than 80 cm [1]. None of the participants reported a history of heart disease, cancer, or any other medically defined disease; none was receiving any medication or taking any antioxidant supplements recently.

Study design

This study was a 4-month double-blind randomized trial. Participants were instructed not to take antioxidant supplements, drugs, and alcoholic beverages during the trial.

The 100 subjects were randomly assigned to VitE1 group (dl-α-tocopheryl acetate, 100 IU/day), VitE2 group (dl-α-tocopheryl acetate, 200 IU/day), VitE3 group (dl-α-tocopheryl acetate, 300 IU/day), or identical-appearing placebo. All subjects were advised to take capsules with meals. VitE capsules and respective

placebos were provided by Weihai Huaxin pharmaceutical factory (Weihai, Shandong, China).

Sample preparation and laboratory analysis

Fasting blood (5 mL) sample was collected from subjects by venipuncture into heparinized tubes at baseline and after 4 months for measurement of biomarkers of oxidative stress, including erythrocyte hemolysis, plasma MDA, and SOD, as well as plasma lipid profile. The samples were transported on dry ice and stored frozen at –80 °C until analysis. Also, anthropometric measurements and plasma glucose were measured for screening MetS at baseline.

Height and weight were measured with the subjects in light clothing and without shoes. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was taken as the minimum circumference at umbilicus level.

Plasma glucose was measured via the glucose oxidase method. Plasma lipid profile, including levels of serum total cholesterol (TC), triglycerides (TG), and HDL- cholesterol (HDL-C), was determined by using standard assay kits (Leadman Bio-Technology, Beijing, China).The units were expressed as mmol/L.

The biomarkers of oxidative stress detected included erythrocyte hemolysis, plasma MDA, and SOD. Erythrocyte hemolysis was detected by peroxide-induced hemolysis test [10]. Erythrocytes were isolated by centrifugation for 20 minutes at 1000 × g at 4 °C. The plasma and buffy coat were removed by aspiration. The cells were washed three times with physiological saline, and finally suspended in an equal volume of physiological saline. This constituted the 1 % (v/v) erythrocyte suspension. The suspension was divided it into A tube and B tube. Two mL of H_2O_2 was added into A tube while 2 mL of distilled water was added into B tube. The mixtures were incubated at 37 °C for 0.5 hour. Then the samples were centrifuged for 5 minutes at 3000 rpm and the supernatant was obtained. Hemolysis was determined by measuring released hemoglobin into the supernatant of the induced samples in a spectrophotometer at 540 nm and was represented by the ratio of the optical density of A tube and that of B tube.

The concentration of malondialdehyde (MDA) was determined using a Jiancheng kit (Jiancheng Bio-Laboratories, Nanjing, China) by colorimetric assay. Briefly, to 0.2 mL of 8.1 % sodium dodecyl sulfate, 1.5 mL of 20 % acetic acid and 1.5 mL of 0.81 % thiobarbituric acid aqueous solution were added in succession. To this reaction mixture, 0.2 mL of the plasma was added. The mixture was then heated in boiling water for 60 minutes. After being cooled to room temperature, 5 mL of butanol: pyridine (15:1v/v) solution was added. The mixture was then centrifuged at 2000 × g for 15 minutes. The upper organic layer was separated, and the intensity of the resulting pink color was read at 532 nm. The level of MDA was expressed as mmol/L.

The activity of superoxide dismutase (SOD) was determined using a Jiancheng kit (Jiancheng Bio-Laboratories, Nanjing, China). Briefly, the method used xanthine and xanthine oxidase to generate superoxide radicals, which reacted with 2-(4-iodophenyl)-3-(4-nitrophenonal)-5-phenyltetrazolium chloride to form a formazan dye. The SOD activity was measured by the degree of inhibition of the reaction. Enzymatic activity was expressed in nitric units in each milliliter of blood plasma. In this method, one nitric unit (NU) meant 50 % of inhibition by SOD of nitric oxide production.

Statistical analysis

All statistical analyses were performed using SPSS 12.0 software. Data are expressed as mean ± SD. Baseline characteristics, levels of oxidative stress, and lipid profiles were compared across all the treatment groups. The mean differences in change over the intervention period between intervention groups and the placebo group were estimated for oxidative stress indices and lipid profile using a general linear model ANOVA. Statistical significance was set at $p < 0.05$.

Results

The trial profile is shown in Figure 1.

Participants' Characteristics

One hundred women with MetS were enrolled. During the trial, 12 women moved out of the trial site or dropped out. In total, 88 women finished the four-month study and none reported any adverse effects related to the interventions administered.

Baseline characteristics including age, BMI, waist circumference, plasma glucose, biomarkers of oxidative stress, and lipid profiles are provided in Table 1. Subjects in the four groups were matched for age and BMI. There were no significant differences in lipid profile, glucose, and biomarkers of oxidative stress among the four groups at baseline ($p > 0.05$).

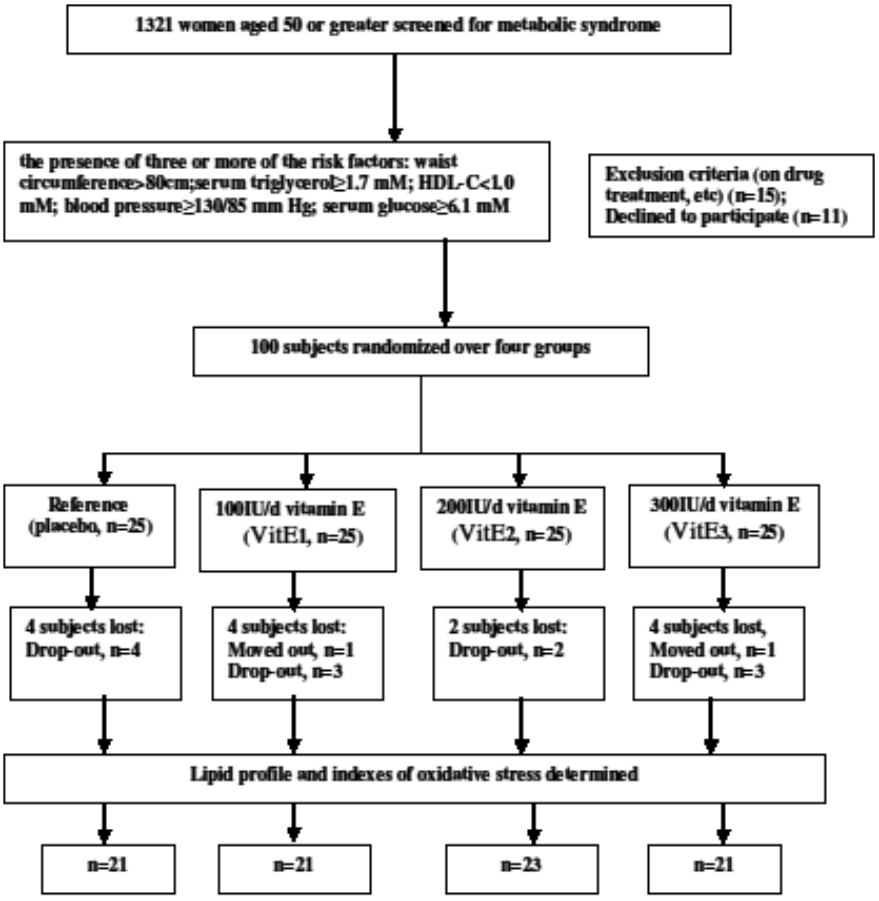

FIGURE 1. The trial profile.
The trial enrollment of women with metabolic syndrome was conducted in Jiaonan' county. At the start of the enrollment, there were 126 women aged 50 or greater who met the inclusion criteria. However, 15 women were on drug treatments or in other situations, and 11 women refused to take any supplements. During the trial, the reasons for subjects lost were moved out of the trial site and drop-out. Some of the subjects did not complete all measurements because of insufficient volume of plasma.

Change of plasma VitE

See Table 2.

After 4 months of trial, the plasma VitE concentrations of 100 IU/day, 200 IU/day, and 300 IU/day supplementation groups were increased by 65 %, 70 %, and 86 % respectively compared with baseline levels. Compared with the placebo, the plasma VitE was significantly changed in the three intervention groups ($p < 0.01$). No significant differences existed among different dosage of vitamin E supplementation groups ($p > 0.05$).

Change of plasma lipidprofile

See Table 2.

In all three VitE supplementation groups, plasma total cholesterol (TC) was decreased by 15 %, 18 %, and 27 % respectively following 4 months of supplementation compared with baseline level. But significant changes were only observed in the VitE2 (200 IU/day) and VitE3 (300 IU/day) groups ($p < 0.05$). No significant differences existed among the different dosages of the vitamin E supplementation groups ($p > 0.05$).

Following 4 months of VitE intervention, plasma HDL-cholesterol levels decreased significantly by 15 %, 13 %, and 18 % respectively in the supplementation groups ($p < 0.05$). As to plasma triglycerides, no

Table I: Characteristics, lipid profile, and indexes of oxidative stress of women with MetS at baseline1

	Placebo		VitE1[2]		VitE2[2]		VitE3[2]	
Characteristics of the subjects	n[3]		n[3]		n[3]		n[3]	
Age, y	21	59.01 ± 3.08	21	58.71 ± 4.06	23	59.00 ± 2.89	21	59.57 ± 4.18
BMI, kg/m^2	21	27.23 ± 1.71	21	26.99 ± 2.19	23	28.09 ± 2.39	21	28.26 ± 2.92
waistline, cm	21	89.95 ± 7.10	21	86.33 ± 6.54	23	89.96 ± 8.50	21	90.33 ± 7.09
Glucose, mmol/L	21	5.28 ± 2.14	21	5.67 ± 2.08	23	5.24 ± 2.35	19	6.34 ± 3.08
Lipid profile indicators[4]								
TC, mmol/L	21	5.19 ± 0.76	21	5.27 ± 0.96	23	5.14 ± 0.80	19	5.39 ± 1.81
HDL-C, mmol/L	21	1.46 ± 0.20	21	1.49 ± 0.28	23	1.49 ± 0.25	21	1.53 ± 0.44
TG, mmol/L	21	1.97 ± 1.12	21	2.49 ± 1.24	23	1.97 ± 0.70	19	2.66 ± 1.43
Oxidative stress indicators								
Hemolysis, %	21	4.18 ± 1.46	21	5.21 ± 2.18	23	5.56 ± 2.50	19	5.51 ± 2.28
Plasma MDA ,mmol/L	21	6.45 ± 1.54	21	6.91 ± 2.05	23	6.65 ± 1.53	18	7.45 ± 1.42
Plasma SOD, NU/mL	21	99.02 ± 10.28	21	102.86 ± 15.83	23	103.00 ± 11.20	18	94.83 ± 13.28

[1] Values are means ± SD
[2] VitE1, 100 IU/day VitE was supplemented in this group, VitE2, 200 IU/day VitE was supplemented in this group, VitE3, 300 IU/day VitE was supplemented in this group.
[3] Total sample size values due to missing values or insufficient plasma analysis.
[4] TC = plasma total cholesterol, HDL-C = plasma high-density lipoprotein cholesterol, TG = plasma triglycerides

significant change was observed in any of the groups ($p > 0.05$).

Change of biomarkers of oxidative stress

See Table 3.

Erythrocyte hemolysis and plasma MDA levels were significantly decreased following different doses of vitamin E supplementation. However, plasma SOD activity was also lowered after the intervention. The level of erythrocyte hemolysis in the three VitE supplementation groups was lowered by 38 %, 46 %, and 62 %, respectively, with plasma MDA levels decreased by 54 %, 52 %, and 70 %.

Although no dose-dependent effect was observed among the three different dosages of VitE supplementation, 300 IU/day VitE supplementation showed the greatest effect. The erythrocyte hemolysis level in this group was significantly lower than that in any other group ($p < 0.05$) and MDA levels were lower in the 100 IU/day VitE group than in the placebo group ($p < 0.05$). At the same time, SOD activity in this group was significantly lower than that of all the other groups ($p < 0.05$).

Discussion

This double-blind intervention trial assessed the effects of three different doses of vitamin E (VitE) supplementation on oxidative stress and lipid profile in MetS. MetS confers an increased risk of the development of diabetes and cardiovascular disease (CVD). Several studies have shown that MetS is associated with increased oxidative stress, which is also postulated to play an important role in the pathogenesis of aging, diabetes, etc.

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the scavenging capacity of the antioxidative defense mechanism of the organism. It has been known for some time that vitamin E prevents oxidative damage by a nonenzymatic process that is regarded as a first line of defense against oxidative damage [11]. As a powerful chain-breaking antioxidant, its role as a therapeutic has been supported by many pathophysiologic, epidemiologic, and mechanistic data. But varied and contradictory results have been reported after VitE trials [5], in some of which VitE had failed to prevent the complications of atherosclerotic disease [12]. Thus, in this study, we provide

Table II: Baseline values, changes, and difference in plasma VitE concentration and lipid profile in four groups after the trial

Indicators[1]	Groups[1]	n[1]	Baseline values	Change[2]	Difference[3]	p-value
					Mean difference (95 % CI)	
VitE, mg/L	placebo	21	6.61 ± 3.02	0.79 ± 2.29	–	–
	VitE1	21	7.75 ± 3.46	5.02 ± 5.35	4.22 (6.84, 1.61)*	0.002
	VitE2	23	6.70 ± 2.20	4.73 ± 3.08	3.93 (6.49, 1.38)*	0.003
	VitE3	21	7.98 ± 3.58	6.91 ± 5.49	6.12 (8.74, 3.51)*	< 0.001
TC, mmol/L	placebo	21	5.19 ± 0.76	0.01 ± 1.29	–	–
	VitE1	21	5.27 ± 0.96	−0.80 ± 0.29	−0.81 (−1.69, 0.06)	0.069
	VitE2	23	5.14 ± 0.80	−0.93 ± 0.26	−0.94 (−1.80, −0.08)*	0.032
	VitE3	19	5.39 ± 1.81	−1.46 ± 0.45	−1.47 (−2.42, −0.52)*	0.003
HDL−C, mmol/L	placebo	21	1.46 ± 0.20	0.05 ± 0.39	–	–
	VitE1	21	1.49 ± 0.28	−0.22 ± 0.21	−0.27 (−0.45, −0.08)*	0.005
	VitE2	23	1.49 ± 0.25	−0.20 ± 0.19	−0.25 (−0.43, −0.07)*	0.007
	VitE3	21	1.53 ± 0.44	−0.28 ± 0.33	−0.33 (−0.51, −0.15)*	0.001
TG, mmol/L	placebo	21	1.97 ± 1.12	0.14 ± 1.58	–	–
	VitE1	21	2.49 ± 1.24	0.24 ± 1.88	0.10 (−1.01, 1.22)	0.852
	VitE2	23	1.97 ± 0.70	0.50 ± 0.91	0.36 (−0.73, 1.46)	0.510
	VitE3	19	2.66 ± 1.43	0.54 ± 2.60	0.41 (−0.74, 1.56)	0.481

[1] See Table 1 for lipid profile indicators, the dosage of VitE supplemented in each group and sample size.
[2] Change: baseline values subtracted from end−of−trial values.
[3] Difference and 95 % CI's are calculated using the placebo group as reference; CI, confidence interval.

novel data with regard to different dosages of VitE supplementation on biomarkers of oxidative stress in MetS women. After 4 months of VitE supplementation, plasma VitE concentrations were increased in subjects, as expected.

Erythrocytes have been used as a model to investigate oxidative damage in biomembranes because of their high vulnerability to peroxidation due to the high, polyunsaturated fatty acid content of their membrane, high cellular oxygen levels, and hemoglobin (Hb) content. They are a constant source of superoxide radicals due to the tendency of hemoglobin to auto-oxidize[13]. Under normal conditions, ROS, which are constantly generated intracellularly, are mostly neutralized by intracellular detoxicants. However, under abnormal conditions, auto-oxidation of hemoglobin is facilitated and an increased flux of superoxide radicals occurs, which may alter unsaturated lipids in erythrocyte membranes and result in a loss of membrane fluidity, followed by cell lysis [14].

Our results, which indicated an decreased erythrocyte hemolysis and hence an improved antioxidant status in the erythrocyte membrane, are in accordance with the report by Odigie *et al.* [15], who found that vitamin E may better protect red blood cells against hemolysis induced by oxidative stress compared to vitamin C. A study carried out by Xie *et al.* suggests that vitamin E inhibits hemolysis induced by microcrystals of monosodium urate monohydrate as a membrane stabilizer [16].

In MetS, increased free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation. MDA, the metabolism product of lipid peroxidation, is a known marker of oxidative stress [17] and can cause a series of changes in cells. We found that VitE supplementation from 100 IU/day to 300 IU/day could decrease plasma MDA concentrations of female patients with MetS, and 300 IU/day was better than 100 IU/day. However, a study [18] performed by Meagher and colleagues in which normal participants were supplemented daily with 200, 400, 800, 1200, or 2000 IU of vitamin E for 8 weeks found no effect of suppression of the lipid peroxidation. We speculate that the difference depends on the

Table III: Baseline values, changes and difference in biomarkers of oxidative stress in four groups after the trial

Indicators	Groups[1]	n[1]	Baseline values	Change[2]	Difference[3]	p-value
					Mean difference (95 % CI)	
Erythrocyte hemolysis, %	placebo	21	4.18 ± 1.46	−0.54 ± 1.71	–	–
	VitE1	21	5.21 ± 2.18	−1.99 ± 2.26	−1.46 (−2.91, −0.003)	0.050
	VitE2	23	5.56 ± 2.50	−2.56 ± 2.72	−2.02 (−3.48, −0.56)*	0.007
	VitE3	19	5.51 ± 2.28	−3.40 ± 2.56	−2.86 (−4.36, −1.37)*	< 0.001
Plasma MDA, mmol/L	placebo	21	6.45 ± 1.54	1.27 ± 1.94	–	–
	VitE1	21	6.91 ± 2.05	−3.72 ± 2.35	−4.99 (−6.19, −3.80)*	< 0.001
	VitE2	23	6.65 ± 1.53	−3.47 ± 1.71	−4.74 (−5.91, −3.57)*	< 0.001
	VitE3	18	7.45 ± 1.42	−5.20 ± 1.45	−6.47 (−7.70, −5.24)*	< 0.001
Plasma SOD, U/mL	placebo	21	99.02 ± 10.28	−0.89 ± 11.49	–	–
	VitE1	21	102.86 ± 15.83	−35.30 ± 15.76	−34.41 (−44.87, −23.96)*	< 0.001
	VitE2	23	103.00 ± 11.20	−41.45 ± 19.46	−40.56 (−50.78, −30.34)*	< 0.001
	VitE3	18	94.83 ± 13.28	−51.56 ± 18.52	−50.67 (−61.58, −39.76)*	< 0.001

[1] See Table 1 for the dosage of VitE supplemented in each group and sample size.
[2] Change: baseline values subtracted from end- of-trial values.
[3] Difference and 95 % CI's are calculated using the placebo group as reference; CI, confidence interval.

original level of oxidative stress in the individuals. In the case that the oxidative stress level is elevated, as in this study, VitE supplementation can have a positive effect.

SOD, one of the most important enzymes in the body's antioxidant defense system, catalyzes the conversion of superoxide anion radicals, which is the first product of oxygen radical formation, to H_2O_2 and hence reduces the toxic effects caused by this radical or other free radicals derived from secondary reactions. However, the decreased activity of superoxide dismutase (SOD) after various doses of VitE supplementation is opposite to our hypothesis. Previous data about the regulation of SOD activity by vitamin E are conflicting. In accordance with our study, Yerer and Aydogan [19] found that SOD activity was significantly reduced after administration of VitE in rats oxidatively stressed by sodium nitroprusside. Also, Dandapat *et al.* [20] discovered a dose-dependent decrease in the SOD activity of hepatopancreatic tissues by VitE in normal freshwater prawns. However, Shirpoor *et al.* [21] found that VitE consumption elevated SOD, and in an animal study, the SOD suppression was reversed by a dose-dependent increase in VitE [22]. In other studies study, SOD activity was not different after VitE supplementation [23].

What is the mechanism underlying the decreased activity of SOD? In an *in vitro* model, Huang *et al.* [24] showed that VitE increased the level of SOD mRNA after 2 days, which was followed by a decrease after 7 days. We assume that the earlier time-course changes in the SOD activity had been bypassed and when we measured at the endpoint after 4 months, we found the decreased activity of SOD. And some authors have considered that SOD activity is up-regulated by an initial dose of VitE and becomes negatively affected by the accumulation of VitE. Another possible mechanism is that VitE might regulate the activity of SOD by an effect on the gene expression of erythrocyte precursors in the bone marrow. Further research should be performed concerning the mechanism of the change of SOD activity.

The level of serum lipids is usually elevated in insulin resistance and such an elevation represents a risk factor for coronary heart disease. In our study, VitE has a positive effect on total plasma cholesterol (TC), which decreased by nearly 20 % compared to the baseline value. But as to high-density lipoprotein cholesterol (HDL-C), an adverse effect was found. In an insightful epidemiological analysis of risks related to HDL-C [25], an increment of 0.03 mmol per liter in the HDL level has been associated with a reduction

of 2 to 4 percent in the risk of cardiac events. So, the decreased level of HDL-C may have some negative association with the risk of cardiac events. In accordance with our study, it has been found that vitamin E may blunt the increase in high-density lipoprotein [26] and diminish the effects of niacin on increasing HDL-C [27]. As to plasma triglyceride, no change was found after the trial. In contrast to our study, some authors considered that vitamin E appeared to protect cells against oxidation without modulating lipid values [14]. A study of 3200 IU/day vitamin E supplementation for 20 weeks also found that the serum lipid profile was not different after the trial [28]. However, Lucas discovered that feeding 525 IU VitE/kg of diet could reduce liver triglycerides in the rat [29]. From a mechanistic point of view, we have limited data to explain the reason for lowered HDL-C in the plasma of MetS women after VitE supplementation, which suggested that either lipid biosynthesis was increased or lipid clearance was decreased. We consider that VitE has a complex effect on plasma lipid metabolism, which is worthy of further research.

The optimal dose of antioxidants remains controversial. It has been considered that vitamin E is safe in doses up to 1,000 mg/day [30] and 800 IU of vitamin E has been suggested to be the effective threshold dose [12]. A dose-response meta-analysis of cohort studies [31] reported in 2008 revealed that supplement uses of vitamin E have an inverse association with CHD risk, with each 30 IU/day increase in vitamin E yielding an estimated overall relative risk for CHD of 0.96 (95 % CI, 0.94–0.99), and it has been suggested that the vitamin E doses in the randomized trials were too low to show any effect by some researchers [32]. However, it has been suggested that high doses of vitamin E (> 400 IU/day) may increase the risk of all-cause mortality [9] and heart failure [33]. Also, some *in vitro* studies suggest that VitE may be a pro-oxidant at certain concentrations. But our data, and those of others [34], suggest that over a wide dose range this is not the case *in vivo.* Although no dose-dependent effect was observed in our study, it hints that 300IU/day VitE might be the most effective dosage for reducing oxidative stress in MetS.

Therapeutic lifestyle changes have been recommended as the primary treatment strategy for MetS. Dietary micronutrients with antioxidant potential could be alternative strategies to ameliorate the symptoms of MetS. In conclusion, our study demonstrates an *in vivo* regulation of oxidative stress by VitE from 100 IU to 300 IU per day, which might have important implications in the prevention and treatment of MetS and the related oxidative stress biology. Further research is needed to identify potential protective mechanisms or utilization of vitamin E in metabolic syndrome.

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